

medium¹⁶. In view of these findings, it is possible to suggest an allosteric function for the external Na⁺ in the transport systems for all these molecules and for NA.

Résumé. L'étude de l'accumulation de la noradrénaline marquée par des coupes de cortex cérébral de rat, montre l'existence de deux systèmes de capture. Seul le

Km du système à haute affinité est affecté par l'exclusion partielle des ions Na⁺ du milieu d'incubation (Na⁺ = 75 mM) par la choline.

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Variation in the Total Content of Alkaline RNase in Mouse Lymphocytes from Different Organs

Wide variations in the alkaline ribonuclease (RNase) activity of different populations of lymphocytes were recently reported by MANSSON et al.¹ in a group of 20 patients with chronic lymphocytic leukemia in whom the total RNase content per cell varied by a factor of 20. In experimental animals, the content of alkaline ribonuclease of homogenates from thymus, spleen and lymph nodes is raised many times following a variety of stimuli which include irradiation by X-rays of the whole body^{2,3}, irradiation of the head only^{3,4}, injection of cortisone and other hormones^{5,6} and antigenic stimulation^{7,8}. In our studies, the value of RNase/mg of DNA in control animals was higher for spleen than for thymus^{3,6} and it seemed that there were variations in RNase content between different populations of lymphoid cells. KRAFT and SHORTMAN⁹ had reported that peritoneal cells of rats contained much more alkaline RNase than lymphocytes from lymph nodes and spleen, and they attributed this to the presence of macrophages. However, the results of the present investigation show that lymphocytes from the peritoneal cell population have higher content of RNase than the macrophage, and that by comparing the RNase content of adherent and non-adherent cells from the thymus, lymph nodes and peritoneal cell population, of normal mice and mice treated with cortisone, subpopulation of lymphocytes which are exceptionally rich in RNase were identified. Previously we¹⁰ found that, at the end of the regenerative phase following partial hepatectomy, the RNase content of livers rose sharply for a few days, and we proposed that RNase was involved in the control of cell division. A similar suggestion was subsequently made by KRAFT and SHORTMAN⁹, and it is tempting to speculate that the difference in the RNase content of lymphocytes from different sites may also be related to the time at which they had undergone cell division.

Materials and methods. Random bred mice, 10–12 weeks old, were used. Peritoneal cells, thymuses and mesenteric

lymph nodes were pooled from 5 animals per experiment. In the cortisone-treated groups, 5 mg of cortisone/mouse (Frederiksberg, Copenhagen) were injected s.c. either 1, 2 or 3 days prior to the removal of the lymphoid cells. Peritoneal cells were obtained without prior stimulation^{11,12}. Peritoneal thymus and mesenteric lymph nodes cell suspensions were prepared at 10⁶ cells/ml. Separation of adherent and non-adherent cells was performed according to EVANS and ALEXANDER¹¹. 2 ml of the suspension of cells derived from peritoneum, thymus or lymph nodes were cultured either in a 3.5 × 6 mm Falcon plastic petri dish or in a siliconized glass tube in medium M199. After the separation, these suspensions were diluted 1:1 with distilled water, and the estimation of total RNase content of cells was carried out as described previously³.

Results and discussion. Table I shows that the RNase content of cells from the peritoneum is very much greater than that from the thymus and lymph nodes. The

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Table I. Ribonuclease activity of cell suspension from the peritoneum, thymus and lymph node cells of normal and cortisone-treated mice

Treatment of mice	RNase ^a activity of cells ^b from		
	Peritoneum	Thymus	Lymph nodes
None	12.2 ± 2.1	0.56 ± 0.2	1.7 ± 0.5
1 day following cortisone ^c	99.0 ± 24.0	5.0 ± 2.9	2.1 ± 0.8
2 days following cortisone	102.0 ± 32.3	2.1 ± 0.6	3.8 ± 1.9
3 days following cortisone	26.6 ± 10.7	4.1 ± 2.2	3.4 ± 1.1

^a Expressed as 10⁻¹⁵ g of crystalline pancreatic RNase per cell. ^b Cells were pooled from 5 mice for each of the 3 experiments (mean of 3 separate experiments ± S.D.). ^c 5 mg cortisone acetate (Fredriksberg Chemical Laboratories Ltd., Copenhagen) given s.c.

Table II. Differences in the ribonuclease activity of adherent and non-adherent cells cultured from the peritoneum and the thymus of normal and cortisone-treated mice

Cells obtained from	RNAse content of cells after 2 h in culture ^{a, b}	
	Adherent cells	Non-adherent cells
Peritoneum from normal mice	12.0 \pm 5.0	28.0 \pm 8.8
From mice 1 day after cortisone ^c	13.2 \pm 4.4	255.3 \pm 68.1
From mice 2 days after cortisone	10.8 \pm 2.7	249.0 \pm 49.5
From mice 3 days after cortisone	14.4 \pm 4.6	56.0 \pm 15.1
Thymus from normal mice	0.6 \pm 0.3	1.6 \pm 0.5
From mice 1 day after cortisone	0.54 \pm 0.3	14.4 \pm 5.5
From mice 2 days after cortisone	0.60 \pm 0.2	7.2 \pm 2.8
From mice 3 days after cortisone	0.63 \pm 0.1	11.2 \pm 5.4
Lymph node from normal mice	1.5 \pm 0.5	5.0 \pm 1.9

^a Expressed as 10^{-15} g of crystalline pancreatic ribonuclease per cell. ^b Cells were pooled from 5 mice for each of the 3 separate experiments (mean of 3 separate experiments \pm S.D.). ^c 5 mg cortisone acetate (Frederiksberg Chemical Laboratories Ltd., Copenhagen) given s.c.

possibility that this difference can be ascribed to the presence in the peritoneal cells of a much higher proportion of phagocytic cells than are found in suspension of cells from the thymus and lymph nodes, was tested by removing glass adherent cells by culturing these cells in plastic petri dishes or in siliconized glass tubes. Similar results were obtained in both types of vessel, and the data shown in Table II are for cells maintained in plastic vessels. The difference in RNAse content is very great, the non-adherent cells from the peritoneum contain some twenty times as much RNAse than do those from the thymus.

The non-adherent cultured cells from both peritoneum and thymus contain more RNAse (see Table II) than the average of the cells before separation, the values for which are shown in Table I. The non-adherent cells recovered after culturing are, by morphological criteria, mostly composed of lymphocytes, yet their RNAse content varies widely and suggests that different populations of lymphocytes may be distinguished on the bases of the amount of alkaline RNAse they contain.

We have shown previously³ that after high doses of cortisone (2.1 mg per mouse), which leads to a marked loss of lymphoid cells from the thymus and lymph nodes, the RNAse content expressed per mg of tissue rises sharply. The data shown in Table I confirm these results but show in addition that the already high RNAse content of the peritoneal lymphocyte is raised still further by treatment of the mice with cortisone. The results in Table II show that cortisone does not significantly alter the RNAse content of the adherent cells.

There is a $160\times$ difference between the RNAse content of non-adherent cells from the peritoneum of cortisone-treated mice as compared with that of the non-adherent cells from the thymus of normal mice. In terms of θ marker, the proportion of T-cells will be

greatest for the thymus and less in the lymph nodes and peritoneal cells¹³.

The RNAse content of the adherent cells from the peritoneum is considerably greater than that of the adherent cells from thymus and lymph nodes, but this data is difficult to interpret. While the adherent peritoneal cells are made up of at least 95% of macrophages (the peritoneal cells when taken from a non-stimulated cavity contain approximately equal numbers of lymphocytes and mononuclear phagocytes¹⁴), the nature of the relatively few adherent cells separated from the thymus and lymph nodes is uncertain. It is noteworthy that cortisone pre-treatment does not affect the RNAse content of any of the adherent cell populations studied.

Zusammenfassung. Die Aktivität der alkalischen Ribonuklease in Peritonealzellen bei Mäusen ist 21- bzw. 7mal stärker als die Aktivität in Thymus- und Lymphknotenzellen. Die meisten ribonukleasereichen Peritonealzellen haften nicht an plastischen Oberflächen. Die Ribonuklease-Aktivität von nichtadhärenten Zellen wird erhöht, wenn Mäuse mit Cortison behandelt werden, und diese Wirkung ist besonders auffallend im Gesamtbestand der peritonealen Zellen.

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¹³ M. C. RAFF and J. J. T. OWEN, *Eur. J. Immun.* 1, 27 (1971).

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Evidence for the Allosteric Nature of IAA Oxidase System in *Phaseolus mungo* Hypocotyls

The synergistic effect of sodium metabisulfite with IAA in the production of adventitious roots in hypocotyl cuttings of *Phaseolus mungo* reported earlier from this laboratory¹ lends support to the view that IAA effects are caused through IAA oxidation products²⁻⁵. This is contrary to the view of other workers⁶⁻⁸, who consider that IAA oxidase causes detoxification of IAA in the plant system.

In in-vitro-experiments that are carried out to determine the activity of IAA oxidase in tissue homogenates, the concentrations of IAA that are used as substrate are fairly high (10^{-4} M). If the destruction of IAA in vivo also occurs at this rate, the plant tissue will be depleted of its endogenous IAA within 1-10 min, as its biosynthesis is considered to proceed at a very slow rate⁹. As the maintenance of a proper balance between the synthesis and